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(54) Title: METHOD FOR THE TREATMENT, PREVENTION OR MINIMIZATION OF HAIR LOSS

(57) Abstract

A composition and method for the treatment, prevention or minimization of hair loss that provides for the topical administration of an effective amount of lipid soluble thioester or thioether of N-acetylcysteine.

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Method for the Treatment, Prevention or Minimization of Hair Loss

This invention is a method for the treatment of alopecia (hair loss) by the topical administration of a thioester or thioether of N-acetylcysteine or a derivative or a salt thereof.

This application is a continuation-in-part of: (i) U.S.S.N. 08/221,856 entitled "Method for Delivering Biologically Active Materials Using a Thioester or Thioether Prodrug", filed on April 1, 1994; (ii) U.S.S.N. 08/175,959 entitled "Method for the Treatment of Traumatic Injury or Disorders Involving Pathogenic Oxidation Pathways Which Affect the Central Nervous System or the Eye", filed on December 30, 1993; (iii) U.S.S.N. 08/147,864, entitled "Topical Application of a Lipid Soluble Thioester or Thioether of N-acetylcysteine for the Treatment of Pathological Conditions Associated with Immune Responses or Inflammatory Conditions", filed on November 4, 1993; (iv) U.S.S.N. 08/131,892 entitled "Method for Treating Diseases Mediated by Proteases", filed on October 5, 1993; (v) U.S.S.N. 08/102,617 entitled "Method for Treating Diseases Characterized by Hyperkeratosis", filed on August 5, 1993; and (vi) U.S.S.N. 08/079,645 entitled Method for the Treatment of Diseases Mediated by Proteases", filed on June 18, 1993.

Background of the Invention

The three major types of hair are: (i) light, soft, thin fetal hair (lanugo hair) which is replaced (ii) by soft and short hair (vellus hair) particularly on the skin surface in humans and (iii) coarse hair (terminal hair) notably occurring on the scalp, eyebrows, eyelashes, and pubic areas (post-puberty). Alopecia is defined as the loss, miniaturization, involution, or increased fragility of the hair at any hair bearing site (i.e., scalp, face (bearded area), eyebrows, eyelashes or body). Alopecia can be caused by many internal and external factors, including genetic factors, chemotherapy, burns, stress, infections, cutaneous and systemic diseases, ionizing radiation exposure, and other unknown factors. In many of these disorders, hair loss is thought to result at least in part, from the formation of free radicals which, by producing oxidative damage, disrupt the hair's normal growth cycles.

The life cycle of hair consists of three phases. In humans, the growing phase, anagen, usually lasts about 1000 days. The resting phase, which lasts approximately 100 days in humans, is called telogen. The third phase is the dying phase, named catagen. The length of hair is genetically determined and is dependent upon both the duration of anagen and the rate of hair growth, human scalp hair usually averages 1 cm per month. Approximately 10 percent of the 100,000 hair follicles on the scalp are in telogen phase at any given time. Therefore, typically no more than 100 hairs are ordinarily shed per day

(Birnbaum, P.S. in Manual of Clinical Problems in Dermatology, First Edition, Olbricht, S.M., Bigby, M.E., Arndt, K.A. eds. Little, Brown and Co., Boston: 1992, pg. 114).

Alopecia is generally divided into androgenetic alopecia, telogen effluvium (shed), anagen effluvium, and alopecia areata. The most common form of alopecia is androgenetic alopecia. Synonyms for this disorder include common baldness, hereditary baldness, male-pattern baldness, premature baldness, and diffuse alopecia. It is a genetically determined form of gradual hair loss that begins as a receding hair line and/or thinning at the vertex (crest) of the scalp. A gradual decrease in hair shaft length and diameter may eventually result in near total replacement of terminal hair with soft, short vellus hair. A band of hair is usually retained along the peripheral scalp. In men, onset can begin as early as adolescence. In women, the pattern is exhibited as thinning of the parietal and vertex regions, and the age of onset is usually two decades after the onset in men.

A topical solution of minoxidil is available for the treatment of androgenetic alopecia. However, the utility of this treatment is modest at best. After four months of treatment, approximately 25%, 7%, and 0.7% of patients achieve minimal, moderate and dense regrowth of hair, respectively. The response appears to be more favorable in women and men under 40, in those who have been bald for less than 10 years, and when the affected area is less than 10 cm in diameter (Price, V.H., Rogaine in the

management of male-pattern baldness and alopecia areata. Proceedings of a Symposium, J. Am. Acad. Dermatol., 1987, Vol 16, pp. 749-750). Patients with severe hair loss are currently instructed that most therapeutic attempts to treat hair loss are ultimately unsuccessful, and physicians must simply help patients make the psychological adjustment to permanent hair loss.

Telogen effluvium is the second most common form of alopecia. It can be induced by such factors as childbirth, surgery, psychological stress, certain drugs, androgen excess, nutritional and metabolic disorders, autoimmune diseases, chronic infections, chronic scalp disease and chronic alopecia areata. Telogen effluvium usually affects less than 20 to 35 percent of the scalp hair, and occurs approximately 6 weeks to 3 months after a triggering event.

Anagen effluvium usually affects more than 80-90 percent of the total body hair, and begins 1 to 2 weeks after a triggering event. Anagen effluvium can be caused by anti-coagulants, anti-metabolites, cytotoxic agents, alkylating agents, and alopecia areata, or by poisoning with lead, arsenic, mercury, thallium, and certain chemotherapeutic agents as well as ionizing radiation.

Alopecia areata, a non-cicatricial (non-scarring) alopecia, is a relatively common disorder of unknown cause or causes. It is characterized by well defined areas of total hair loss, typically affecting the scalp, although it may extend to effect the entire scalp or even hair follicles on the entire

body. Alopecia areata occurs in patients with atopy or Down's syndrome, and usually occurs before age 25. The typical lesions usually appear over a 24 hour period as 3-4 cm asymptomatic smooth bald patches, commonly on the scalp. However, the beard, eyebrows, and/or eyelashes may also be affected. The course of the baldness is variable, and some patients experience regrowth of hair within one year. One third of the patients never experience hair regrowth, and many suffer from recurrences of areas of baldness.

High potency corticosteroids may be prescribed for topical or intralesional treatment of alopecia areata. Topical therapies are often ineffective and intralesional therapy is impractical. The effectiveness of this approach is questionable, however, and most patients who respond experience a recurrence of hair loss upon discontinuation of therapy. Moreover, any benefits of this therapy usually are outweighed by its side effects.

An alternative therapy is the combination of Psoralen and ultraviolet radiation treatments (photochemotherapy, PUVA). This therapy has had some effect in certain severe cases of alopecia areata. However, the inconvenience and long-term toxicity and low rate of response of this treatment make it impractical for most patients. Furthermore, as treatments are successful, hair grows in, preventing effective penetration of light and therefore limiting the effectiveness of subsequent treatments.

The induction of irritant dermatitis or allergic contact dermatitis in areas affected by alopecia areata can sometimes promote hair regrowth. However, the local pain and discomfort associated with this therapy renders it essentially useless.

In addition to the four forms of hair loss already discussed, a wide range of inflammatory disorders can cause hair loss as a result of scarring that produces complete destruction of the pilosebaceous unit. These disorders include discoid lupus erythematosus (DLE), lichen planopilaris (LPP), morphea, cicatricial pemphigoid, and follicular mucinosis. Basal cell carcinoma, infections (e.g., herpes zoster, leprosy), and physical injury (e.g., burns, radiodermatitis) may also result in scarring and hair loss.

Radiation and chemotherapy treatments for cancers may also cause alopecia, which may be transient or permanent. The loss of hair due to radiation therapy is dose dependent, but usually occurs in the range of 200-800 rads. Follicles in the anagen phase are about 3 times more sensitive to this disruption than are follicles in the telogen phase. Free radicals generated as part of the cytotoxic mechanisms of action of chemotherapeutic agents and ionizing radiation are thought to have a major role in the hair loss associated with these modalities of cancer therapy.

Cytotoxic drugs may also produce partial or complete inhibition of mitosis or impairment of metabolic processes in the hair matrix, resulting in a thinned, weakened hair shaft. This

form of alopecia only affects hairs in the anagen phase of growth, and is therefore classified as a type of anagen effluvium. Hair loss due to chemotherapy is most pronounced in the scalp. Other terminal hairs, such as facial or pubic hairs, are variably affected. Hair loss is usually first observed 1-2 weeks after initiation of chemotherapy, but becomes progressively more marked 1-2 months later.

Thiele notes that medically and cosmetically acceptable inhibitors of lipid-peroxidation may have value in reducing hair loss. However, these antioxidants produce numerous side effects when applied to the skin and they are further limited in their ability to penetrate the epidermis (Thiele, F.A.J. British J. of Dermatology, 1975, Vol. 92, pg. 355).

U.S. Patent No. 2,986,573 describes a method to promote hair growth that includes the topical application of 6-chloro-3-dimethylaminoethoxymethyl-2H-1,2,4-benzothiadiazine 1,1-dioxide and 6-chloro-3-cyclohexenyl-3,4-dihydro-2H-1,2,4-benzothiazine 1,1-dioxide in DMSO or a suspension.

U.S. Patent Nos. 4,139,619 and 4,596,812 describe a process for stimulating the growth of mammalian hair by the application of 6-amino-4-(substituted amino)-1,2-dihydro-1-hydroxy-2-iminopyrimidines in combination with a topical pharmaceutical carrier.

Most current treatments for the common or uncommon alopecias are either not effective or have unfavorable side effects that render them unsuitable for the treatment of a

cosmetic condition. Several substances are known to be effective when administered systemically, but the increased hair growth is not limited to the target areas and many such agents have unacceptable side effects when administered systemically. Accordingly, an effective topical therapy which could prevent or lessen the extent of the hair loss, or which could promote regrowth of hair, would be valuable in serving the cosmetic and psychological needs of subjects with alopecia, while avoiding the introduction of a secondary systemic drug which could interfere, for example, with chemotherapy or lessen the effectiveness of anti-cancer agents.

Kim et al. studied the effect of topically applied N-acetylcysteine on the reduction of skin reactions caused by radiation therapy (Kim, J-A. et al, Seminars in Oncology, 1983, Vol 10, Suppl. 1, pp. 86-88). Six patients received 4500 rad/4 weeks to the area of the axilla and four patients received 5000 rad/5 weeks to the whole pelvic region. Prior to radiation therapy, the researchers applied gauze soaked in a 10% aqueous N-acetylcysteine solution to the areas of skin which were to be irradiated. In comparison to twelve matched control patients, the results illustrated that N-acetylcysteine lessened the degree of moist desquamation caused by the therapy, decreased the time needed for wound healing, and decreased the need for analgesics to control pain associated with the skin reaction. However, pre-treatment with N-acetylcysteine did not eliminate the radiation dermatitis. The authors also assert that animal experiments

suggest that topical application of radioprotective agents can prevent hair loss from radiation therapy, although no experimental data were presented. One interpretation of the results of Kim et al. is that pretreatment with aqueous N-acetylcysteine was unable to prevent desquamation because the compound was unable to penetrate intact stratum corneum, yet, once the skin had been partially damaged, the N-acetylcysteine was absorbed into the skin and helped to protect it from further radiation induced damage.

Jiminez, et al., examined the effect of N-acetylcysteine in preventing the alopecia induced by the chemotherapeutic agents cyclophosphamide, cytarabine or adriamycin (Jiminez, J. J., et al., Cancer Investigation, 1992, Vol. 10(4), pp. 271-276). The results of this study illustrate that the sub-cutaneous injection of N-acetylcysteine can effectively prevent cyclophosphamide induced alopecia. However, N-acetylcysteine did not prevent the alopecia when it was applied topically in the same alopecia model. Administration of N-acetylcysteine in conjunction with a biological response modifier ImuVert (a membrane vesicle-ribosome preparation derived from the bacterium Serratia marcescens, sold by Cell Technology, Inc., Boulder, Colorado) may have enhanced the penetration of N-acetylcysteine into the skin resulting in slight prevention of alopecia.

These results demonstrate that N-acetylcysteine is effective in treating chemotherapeutically-induced alopecia when

applied below the stratum corneum (e.g. when injected subcutaneously). N-acetylcysteine (a charged amino acid derivative), however, is probably not suitable for topical treatment of alopecia because it is insufficiently lipid soluble to penetrate through the stratum corneum (outer most layer of skin). Due to this, an effective means of topical delivery of N-acetylcysteine through the stratum corneum could be of enormous therapeutic benefit.

It is therefore an object of the present invention to provide a method for the treatment of alopecia.

It is another object of the present invention to provide a pharmaceutical composition that contains an active ingredient that can readily pass through the skin and, in particular, can readily pass through the stratum corneum, for effective treatment of alopecia.

Summary of the Invention

One embodiment of the present invention is a pharmaceutical composition for the topical treatment of alopecia which contains an effective amount of a lipid soluble thioester or thioether of N-acetylcysteine or a pharmaceutically acceptable salt or derivative thereof.

Another embodiment of the present invention is a method for treating alopecia via the topical administration of a lipid soluble thioether or thioester of N-acetylcysteine or its

pharmaceutically acceptable salt or derivative optionally in a pharmaceutically acceptable carrier to induce regrowth of hair, and/or to prevent further loss of hair.

The thioesters and thioethers disclosed herein represent a new prodrug form of N-acetylcysteine which facilitate the transdermal or topical delivery of N-acetylcysteine for treatment of alopecia.

The thioester derivatives of N-acetylcysteine are stable in non-biological aqueous solutions such as saline, phosphate buffered saline, lactated Ringer's solution, sterile water and water-containing creams, gels, lotions, foams, and suspended particles. However, in biological fluids such as plasma or tissue samples, the thioester of N-acetylcysteine is cleaved, releasing N-acetylcysteine, which exhibits antioxidant properties.

The delivery of N-acetylcysteine through the skin in the form of a thioester or thioether is useful in the treatment, prevention or minimization of all types of alopecia, including androgenetic alopecia, alopecia areata, telogen effluvium, anagen effluvium, and other, non-specific types of hair loss which are caused by oxidative damage.

Brief Description of the Drawing

Figure 1 is a graph of the percentage of S-lauryl-N-acetylcysteine remaining over time in (a) a solution

of 80% PBS and 20% methanol; and (b) a solution of 80% PBS and 20% methanol containing skin fragments.

Detailed Description of the Invention

The term alkyl, as used herein, refers to a saturated straight, branched or cyclic (or a combination thereof) hydrocarbon of C_1 to C_{22}, and specifically includes methyl, ethyl, propyl, isopropyl, cyclopropylmethyl, cyclobutylmethyl, butyl, isobutyl, t-butyl, pentyl, cyclopentyl, isopentyl, neopentyl, hexyl, isohexyl, cyclohexyl, 3-methylpentyl, 2,2-dimethylbutyl, 2,3-dimethylbutyl, heptyl, octyl, nonyl, and decyl.

The term aryl or aromatic, as used herein, refers to phenyl, or substituted phenyl, wherein the substituent is halo, alkyl, alkoxy, alkylthio, haloalkyl, hydroxyalkyl, alkoxyalkyl, methylenedioxy, cyano, C(O)(alkyl), carboxylic acid, CO_2alkyl, amide, amino, alkylamino or dialkylamino, and wherein the aryl group can have up to 3 substituents.

The term aralkyl refers to an aryl group with an alkyl substituent.

The term halo refers to chloro, fluoro, bromo and iodo groups.

The term alkaryl refers to an alkyl group with an aryl substituent, including benzyl, substituted benzyl, phenethyl or substituted phenethyl, wherein the substituents are as defined above for aryl groups.

The term alkoxyalkyl refers to an alkyl group joined to another alkyl group through an oxygen atom. Non-limiting examples of this group include methoxymethyl.

The term aryloxyalkyl refers to an aryl group joined to an alkyl group through an oxygen atom. Non-limiting examples of this type of group include phenoxymethyl.

The term acyloxyalkyl refers to an acyl group (aliphatic-C(O))- which is linked to an alkyl group through an oxygen atom.

As used herein, the term amino acid refers to an aliphatic compound containing both an amino group and a carboxylic acid group. Non-limiting examples of suitable amino acids include alanyl, valinyl, leucinyl, isoleucinyl, prolinyl, phenylalaninyl, tryptophanyl, methioninyl, glycinyl, serinyl, threoninyl, cysteinyl, tyrosinyl, asparaginyl, glutaminyl, aspartoyl, glutaroyl, lysinyl, argininyl, and histidinyl.

As used herein the term fatty acid refers to a long chain (C_6 to C_{24}) aliphatic carboxylic acid. Non-limiting examples of suitable fatty acids include lauric, oleic, caproic, linoleic, linolenic, caprylic, capric, perlargonic, neononanoic, neodecanoic, palmitelaidoic, myristic, palmitic, stearic, arachidic, behenic, lignoceric, heptanoic, nonanoic, undecanoic, tridecanoic, pentadecanoic, heptadecanoic, nonadecanoic, heneicosanoic, tricosanoic, arachidonic, docosahexanoic, elaidic, erucic, nervonic, palmitoleic or petriselinic acid, undecylenic and other trans fatty acids.

As used herein, the term α-hydroxy acid refers to aliphatic carboxylic acid which contains a hydroxy group on the carbon adjacent to the carboxylic acid moiety. Non-limiting examples of suitable α-hydroxy acids include lactic and glycolic acid.

As used herein, the term aromatic or alkyl dicarboxylic acid refers to an alkyl or aryl moiety which is substituted with two carboxylic acid groups. Non-limiting examples of suitable dicarboxylic acids include azelaic acid, sebacic acid, phthalic acid, terephthalic acid, isophthalic acid, adipic acid, 1,10-dodecanoic acid, fumaric acid, 1,4-diphenylenediacrylic acid, azeleic acid, pimelic acid, suberic acid, itaconic acid, biphenyl-4,4'-dicarboxylic acid, benzophenone-4,4'-dicarboxylic acid, hydroquinone-O,O-diacetic acid, 2,2-bis-(4-hydroxyphenyl)propane-O,O-diacetic acid, 1,4-phenylene-dipropionic acid, and cyclohexane dicarboxylic acid.

As used herein, the term residue, for example of an acid, refers to the remainder of the named molecule after the thioester or thioether is formed with the N-acetylcysteine. For example, the residue of a carboxylic acid would be the remainder of the relevant molecule except for the -OH moiety of the carboxylic acid which was displaced during the formation of the thioester or thioether.

The term biologically active molecule, as used herein, refers to a molecule which exhibits a biological activity, for example, as an antioxidant or a free radical inhibitor. Non-

limiting examples of this type of molecule include vitamin E, ascorbic acid, vitamin D, including 1α,25-dihydroxy vitamin D_3, (1α,24,25)-trihydroxy vitamin D_3, 1α-hydroxy vitamin D_3, (1α,25,26)-trihydroxy vitamin D_3, and derivatives thereof. Suitable derivatives of these molecules are well known to those skilled in the art of organic and medicinal chemistry. Non-limiting examples of suitable derivatives include hydroxylated, alkylated, and acylated derivatives of these vitamins.

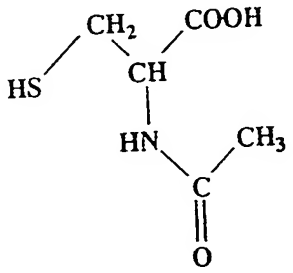
The term aliphatic refers to a hydrocarbon, typically of C_1 to C_{20}, that can contain one or a combination of alkyl, alkenyl, or alkynyl moieties, and which can be straight, branched, or cyclic, or a combination thereof.

The term "enantiomerically enriched composition or compound" refers to a composition or compound that includes at least 95% and optimally 97, 98, 99 or 100% by weight of a single enantiomer of the compound.

I. N-Acetylcysteine and its Derivatives

Cysteine is an amino acid with one chiral carbon atom. It can exist as an L-enantiomer, a D-enantiomer or a racemic mixture of the L and D enantiomers. The L-enantiomer is the naturally occurring configuration. Cysteine is an important substrate in the growth of hair.

N-acetylcysteine (acetamido-mercaptopropionic acid, NAC) is the N-acetylated derivative of cysteine having the formula



It exists as an L-enantiomer, a D-enantiomer, an enantiomerically enriched composition of one of the enantiomers, or a racemic mixture of the L and D enantiomers. Any of these forms of N-acetylcysteine can be delivered as a lipophilic derivative for the treatment, prevention or minimization of hair loss caused by oxidative damage or free radical mediated processes.

In a preferred embodiment, a single isomer or an enantiomerically enriched composition of a thioester or thioether of N-acetylcysteine or its salt is used for the treatment, prevention or minimization of hair loss. A preferred isomer is the naturally occurring L-enantiomer.

N-Acetylcysteine is known to exhibit antioxidant activity (Smilkstein, Knapp, Kulig and Rumack, N. Engl. J. Med. 1988, Vol. 319, pp. 1557-1562; Knight, K.R., MacPhadyen, K., Lepore, D.A., Kuwata, N., Eadie, P.A., O'Brien, B. Clinical Sci., 1991, Vol. 81, pp. 31-36; Ellis, E.F., Dodson, L.Y., Police, R.J., J. Neurosurg., 1991, Vol. 75, pp. 774-779). The sulfhydryl functional group is a well characterized, highly

reactive free radical scavenger. In addition, N-acetylcysteine may behave as an oxygen radical scavenger through a pharmacodynamic pathway.

N-acetylcysteine is also known to promote the formation of glutathione (a tri-peptide, also known as g-glutamyl-cysteinylglycine), which is important in maintaining cellular constituents in the reduced state (Berggren, M., Dawson, J., Moldeus, P. FEBS Lett., 1984, Vol. 176, pp. 189-192). The formation of glutathione may enhance the activity of glutathione peroxidase, an enzyme which inactivates hydrogen peroxide, a known precursor to hydroxyl radicals (Lalitha, T., Kerem, D., Yanni, S., Pharmacology and Toxicology, 1990, Vol. 66, pp. 56-61).

N-acetylcysteine is also an effective mucolytic agent, due to the reactive sulfhydryl group in the molecule (Lightowler, J.E. and Lightowler, N.M., Arch. Int. Pharmacodyn. Ther. 1971, Vol. 189, pp. 53-58). The sulfhydryl group probably opens sulfide linkages in mucus, thereby lowering mucosal viscosity. N-acetylcysteine is also used for the treatment of acetaminophen overdoses (Smilkstein, M.J., Knapp, G.L., Kulig, K.W. and Rumack, B.H., N. Engl. J. Med. 1988, Vol. 319, pp. 1557-1562). A large overdose of acetaminophen results in a larger portion of the drug being metabolized via a free radical (cytochrome P-450) pathway, which in turn results in hepatic cellular necrosis. N-acetylcysteine, when administered within the first few hours of acetaminophen overdose, protects the liver by acting as an

alternate substrate for conjugation with, and detoxification of, the reactive metabolite.

In addition to its mucolytic and free radical scavenging ability, N-acetylcysteine has been reported to be an effective collagenase inhibitor (Lemp, M.A. and Roddy, M., Ann. Ophthalmol. 1974, Vol. 6, pp. 893-895) and an antioxidant *in vivo* (Knight, K.R., MacPhadyen, K., Lepore, D.A., Kuwata, N., Eadie, P.A., O'Brien, B. Clinical Sci., 1991, Vol. 81, pp. 31-36; Ellis, E.F., Dodson, L.Y., Police, R.J., J. Neurosurg., 1991, Vol. 75, pp. 774-779). It has also been reported that N-acetylcysteine can reduce the activity of porcine proteolytic enzymes, leukocyte elastase and pancreatic elastase by greater than 55% in vitro (Morrison, H.M., Burnett, D. and Stockley, R.A., Biol. Chem. Hoppe Seyler 1986, Vol. 367, pp. 177-182). In yet another capacity, N-acetylcysteine can act as an inhibitor of tumor necrosis factor-alpha production *in vivo* (Peristeris, P. et al, Cell. Immunol. 1992, Vol. 140, pp. 390-399).

N-acetylcysteine possesses many other properties which may contribute to its therapeutic benefit in the treatment, prevention or minimization of hair loss. For example, N-acetylcysteine exhibits anti-inflammatory activity (see U.S.S.N. 08/147,864, entitled "Topical Application of a Lipid Soluble Thioester or Thioether of N-Acetylcysteine for Treatment of Pathological Conditions Associated with Immune Responses or Inflammatory Conditions").

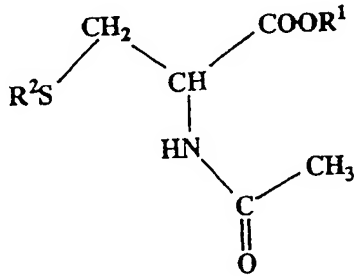
The present invention focuses on two discoveries: first, N-acetylcysteine can be converted into lipid soluble derivatives that are capable of penetrating the stratum corneum, and second, that these lipid soluble derivatives can be broken down *in vivo* to the active parent compound, N-acetylcysteine, at the site of application for treatment of hair loss.

The ester or ether residue of the selected N-acetylcysteine derivative can be either an inert substance or a biologically active substance which itself can have therapeutic benefit for the treatment of alopecia or associated disorders.

As used herein, the term lipophilic thioester or thioether derivative of N-acetylcysteine refers to any thioester or thioether that is capable of passing through the stratum corneum in a therapeutically effective concentration and includes, but is not limited to, either:

(i) any compound that, upon administration to the recipient, is capable of providing directly or indirectly the compounds disclosed herein; or alternatively,

(ii) a compound of the formula:



wherein R^1 is hydrogen, alkyl, aryl, alkaryl, aralkyl, alkoxyalkyl including methoxymethyl, aryloxyalkyl such as phenoxymethyl, an amino acid salt formed by the reaction of the amino group of a naturally occurring amino acid with the carboxylic acid group of the N-acetylcysteine or derivative thereof; an amine salt formed by the reaction of an amine-containing antibiotic with the carboxylic acid group of the N-acetylcysteine, or an inorganic cation including but not limited to sodium, potassium, magnesium, calcium, zinc, bismuth, barium, aluminum, copper, cobalt, nickel, and cadmium; and wherein the amino acid includes but is not limited to alanyl, valinyl, leucinyl, isoleucinyl, prolinyl, phenylalaninyl, tryptophanyl, methioninyl, glycinyl, serinyl, threoninyl, cysteinyl, tyrosinyl, asparaginyl, glutaminyl, aspartoyl, glutaroyl, lysinyl, argininyl, and histidinyl; and

R^2 is alkyl, aryl, alkaryl, aralkyl, alkyloxyalkyl including methoxymethyl, aryloxyalkyl such as phenoxymethyl, C(O or S)alkyl, C(O or S)aryl, C(O or S)alkaryl, C(O or S)aralkyl, C(O or S)alkyloxyalkyl, C(O or S)acyloxyalkyl, phosphate, the residue of a saturated or unsaturated fatty acid, including but not limited to lauric, oleic, caproic, linoleic, linolenic, caprylic, capric, perlargonic, neononanoic, neodecanoic, palmitelaidoic, myristic, palmitic, stearic, arachidic, behenic, lignoceric, heptanoic, nonanoic, undecanoic, tridecanoic, pentadecanoic, heptadecanoic, nonadecanoic, heneicosanoic,

tricosanoic, arachidonic, docosahexanoic, elaidic, erucic, nervonic, palmitoleic or petriselinic acid, the residue of lactic acid, retinoic acid, or ascorbic acid (to form the thioester) or other α-hydroxy acid, or the residue of a dicarboxylic acid (wherein N-acetylcysteine is bound through either or both carboxylic acid groups), including but not limited to cromolyn, nedocrimil, or other mast cell stabilizers, azelaic acid, methotrexate, sebacic acid, phthalic acid, terephthalic acid, isophthalic acid, adipic acid, 1,10-dodecanoic acid, bis(p-carboxyphenoxyalkane), fumaric acid, 1,4-diphenylenediacrylic acid, azeleic acid, pimelic acid, suberic acid (octanedioic acid), itaconic acid, biphenyl-4,4'-dicarboxylic acid, benzophenone-4,4'-dicarboxylic acid, p-carboxyphenoxyalkanoic acid, hydroquinone-O,O-diacetic acid, 1,4-bis-carboxymethyl benzene, 2,2-bis-(4-hydroxyphenyl)propane-O,O-diacetic acid, 1,4-phenylene-dipropionic acid, cyclohexane dicarboxylic acid, branched monomers such as 1,3,5-benzenetricarboxylic acid, the residue of another active molecule such as vitamin D including but not limited to 1α,25-dihydroxy vitamin D_3, (1α,24,25)-trihydroxy vitamin D_3, 1α-hydroxy vitamin D_3, (1α,25,26)-trihydroxy vitamin D_3) and other derivatives of vitamin D_3, including but not limited to hydroxylated, alkylated, and acylated derivatives thereof, and vitamin E.

Non-limiting examples of amine-containing antibiotics that can be used to form a salt with N-acetylcysteine include, but are not limited to erythromycin, propionylerythromycin,

neomycin, gentamycin, tobramycin, kanamycin and mechlocycline, clarithromycin and azithromycin.

N-acetylcysteine that contains a combination of R^1 and R^2 as described herein, as well as combinations of N-acetylcysteine derivatives, can be used for the delivery of N-acetylcysteine to treat, prevent or minimize all types of alopecias.

To counter the harmful effects of oxidative processes, including but not limited to free radical mediated lipid peroxidation, the body naturally produces or utilizes from dietary sources a number of defensive compounds such as α-, β- and γ-tocopherols (vitamin E, which is a known antioxidant), ascorbic acid, β carotene (provitamin A), catalase, superoxide dismutase, glutathione, glutathione peroxidase, glutathione reductase, and other compounds and enzymes. Vitamin E is known to be a scavenger of both lipid peroxyl radicals and oxygen radicals, and to have membrane stabilizing action. Therefore, in one preferred embodiment, a thioester, thioether or ether of N-acetylcysteine is provided in which the thioester, thioether or ether moiety is one of the compounds naturally used by the body to minimize oxidative damage, including, but not limited to an enzyme, vitamin, or other biological molecule with antioxidant properties or that mediates antioxidant processes. If necessary, the material can be linked to N-acetylcysteine through a biodegradable linking moiety, as well as through methods known to those skilled in the art of organic synthesis and biochemistry.

II. Pharmaceutical Compositions of Thioester or Thioether of N-acetylcysteine

Humans, equines, canines, bovines and other animals, and in particular, mammals, with alopecia, and in particular, alopecia caused by oxidative stress or free radicals, arising from either external factors, chemicals, ionizing radiation, or endogenous sources, can be treated by delivery of an effective amount of a pharmaceutically acceptable lipid soluble thioester or thioether of N-acetylcysteine or salt thereof, optionally in a pharmaceutically acceptable carrier or diluent.

As used herein, the term pharmaceutically acceptable salts or complexes refers to salts or complexes that retain the desired biological activity of the above-identified compounds and exhibit minimal undesired toxicological effects. Pharmaceutically acceptable carboxylic acid and mercaptyl salts are known to those skilled in the art, including inorganic salts with cations such as zinc, calcium, bismuth, barium, magnesium, aluminum, copper, cobalt, nickel, cadmium, sodium, potassium, and the like, or with a cation formed with a nitrogenous base such as ammonia, N,N-dibenzylethylene-diamine, D-glucosamine, or ethylenediamine.

In general, the derivatives of N-acetylcysteine disclosed herein are "prodrugs" of N-acetylcysteine, that are either active in the prodrug form or that are cleaved *in vivo* to provide the parent N-acetylcysteine as well as an inert or active ester or ether moiety.

Modifications of the active compound can affect the bioavailability and rate of metabolism of the active species, thus providing control over the delivery of the active species through the stratum corneum. For example, it is well known in the art that various modifications of the active molecule, such as alteration of charge, can effect water and lipid solubility and thus alter the potential for crossing the stratum corneum. Further, the modifications can affect the bioactivity of the compound, in some cases increasing the activity over the parent compound or increasing the permeability of the parent compound through the stratum corneum. This can easily be assessed by synthesizing the derivative and testing its activity according to the methods described herein, or other methods known to those skilled in the art.

Preferred derivatives include, but are not limited to the thioester or thioether of N-acetylcysteine and the residue of oleic acid (S-oleoyl-N-acetyl-L-cysteine), lauric acid (S-lauryl-N-acetyl-L-cysteine), myristic acid (S-myristoyl-N-acetyl-L-cysteine), capric acid (S-caprolyl-N-acetyl-L-cysteine), retinoic acid (S-3,7-dimethyl-9-(2,6,6-trimethyl-1-cyclohexen-1-yl)-2,4,6,8-nonatetraenoyl-N-acetyl-L-cysteine), lactic acid (S-lactoyl-N-acetyl-L-cysteine) and other α-hydroxy acids, ascorbic acid (S-ascorboyl-N-acetylcysteine), vitamin E, vitamin D (including but not limited to 1α,25-dihydroxy vitamin D_3, (1α,24,25)-trihydroxy vitamin D_3, 1α-hydroxy vitamin D_3, (1α,25,26)-trihydroxy vitamin D_3) and other derivatives of

vitamin D_3 including but not limited to hydroxylated, alkylated, and acylated derivatives thereof, or other enzymes, vitamins, or other biological molecules with antioxidant properties or that mediate the antioxidant processes.

The active compound is preferably included in a pharmaceutically acceptable carrier or diluent in an amount sufficient to deliver to a patient a therapeutically effective amount of the drug for any of the above conditions without causing serious toxic effects in the patient treated. If the derivative exhibits activity in itself, the effective dosage can be estimated as above using the weight of the derivative, or by other means known to those skilled in the art.

The concentration of active compound in the drug composition will depend on absorption, distribution, deactivation, and excretion rates of the drug as well as other factors known to those skilled in the art. Dosage values will also vary with the severity of the condition to be alleviated. For any particular subject, specific dosage regimens should be adjusted over time according to the individual need and the professional judgment of the person administering or supervising the administration of the compositions. The concentration ranges set forth herein are exemplary only and are not intended to limit the scope or practice of the claimed composition. The active ingredient can be administered at once, or can be divided into a number of smaller doses to be administered at varying time

intervals. In a preferred embodiment, the N-acetylcysteine prodrug is administered in an amount of 0.001 to 50 mg/kg/day.

The active compound or pharmaceutically acceptable derivatives or salts thereof can also be mixed with other active materials that do not impair the desired action, or with materials that supplement the desired action, such as antioxidants, antibiotics, anti-fungals, anti-inflammatories, disinfectants, or anti-viral compounds.

When administered topically, a topical formulation containing an effective amount of N-acetylcysteine derivative is administered typically in an aqueous solution that can include polymers, aqueous suspension, ointment, gel or cream vehicle. Except ointments, these vehicles may contain liposomes for creating a reservoir of dissolved agent.

III. Synthesis and Chemical Properties of Derivatives of N-Acetylcysteine

Thioester and thioether derivatives of N-acetylcysteine can be easily prepared using standard methods known to organic chemists. A general synthetic route is outlined below.

N-acetylcysteine in dry tetrahydrofuran (THF) is stirred under inert atmosphere. One equivalent of triethylamine is added and the reaction mixture is chilled to 5°C. One equivalent of the desired acid chloride, dissolved in THF, is added slowly to the reaction mixture. After addition, the reaction mixture is stirred for three hours, and reaction

progress monitored by thin-layer chromatography until the reaction approaches completion.

Desired acid chlorides are formed by the addition of an excess of thionyl chloride to the fatty acid (for example) under inert, dry conditions according to methods known to those skilled in the art.

Thioethers can be formed using a variety of synthetic methods known to those skilled in the art, including by the Williamson ether synthesis, where the sodium salt of N-acetylcysteine is formed by treatment with base, and further reacted with an alcohol.

Functional groups can be protected as necessary, as known to those skilled in the art. See, for example, Greene, et al., "Protective Groups in Organic Synthesis," John Wiley and Sons, Second Edition, 1991. For example, the hydroxy group in the α-hydroxy acids must be protected prior to reaction with thionyl chloride, and subsequent reaction with N-acetylcysteine. Once the thioester is formed the hydroxyl group can then be de-protected.

Additionally, the ester and ether derivatives may be synthesized using the carboxylic functional group of the thioester derivative of N-acetylcysteine. In synthesizing these derivatives, it is typically useful first to form a lipid soluble thioester (e.g., S-lauryl-N-acetylcysteine), followed by formation of the ether/ester. First, this protects the sulfhydryl group and prevents it from interfering with the second

reaction. Second, the protection of the sulfhydryl group allows the synthesis to be carried out in a non-aqueous solution.

Specific examples of the synthesis of thioethers of N-acetylcysteine are set forth below.

Example 1 Preparation of N-Acetyl-S-(L)-lactoyl-(L)-cysteine

Synthesis of O-(t-butyldiphenylsilyl-methyl-(L)-lactate (3)

Methyl-(*S*)-(-)-lactate, 1 (0.50 mol, 51.6 g) and imidazole (1.1 mol, 74.4 g) were dissolved in 60 mL of anhydrous THF. The reaction mixture was stirred under N_2 for 10 to 15 minutes until all material was dissolved. To this solution was added, dropwise from a dropping funnel, a solution of *t*-butyldiphenylsilyl chloride, 2 (0.55 mol, 150.3 g) in 110 mL of anhydrous THF. During the addition, the reaction mixture was kept at 5 to 10 °C. The addition took approximately one hour. The reaction mixture was stirred overnight under N_2. The end of the reaction was observed by the disappearance of the methyl-(*S*)-lactate by gas chromatography (R_t 0.7 min., 1; 9.0 mins., 2; 10.5 mins., 3). The reaction mixture was cooled with an ice bath before adding 200 mL of water and 200 mL of ether. The product was further extracted with 2 x 150 mL of ether. The organic layers were combined and washed with 2 x 150 mL of water, 2 x 150 mL of ammonium chloride solution until the pH reached 5, dried with magnesium sulfate, filtered by gravity, and concentrated under reduced pressure. The residue was further pumped under high

vacuum overnight. A pale yellow oil was collected (183.6 g, yield greater than 100%; some silanol by-product from excess silylchloride may be responsible for this excess mass). The oil crystallized upon standing as a white waxy solid. NMR, δ (ppm),(100 MHz, $CDCl_3$: 1.10 (s, 9H); 1.38 (d, J = 5 Hz, 3H); 3.58 (s, 3H); 4.29 (q, J = 5 Hz, 1H); 7.30 - 7.50 (m, 6H); 7.60 - 7.80 (m, 4H).

Synthesis of O-(*t*-butyldiphenylsilyl)-(L)-lactic acid (4) To a solution of O-(t-butyldiphenylsilyl-methyl-(L)-lactate, 3 (0.474 mol, 176.7 g) in 400 mL of methanol was added 400 mL of sodium hydroxide solution (1M). The mixture was heated to 60°C for 2.5 hrs. The solution, originally opaque, became clear. TLC indicated a complete hydrolysis of the ester (R_f 0.6, 3; 0.05, 4, hexane:ether, 7:3). The methanol was evaporated under reduced pressure. The basic aqueous solution was washed with 3 x 300 mL of ether to remove silanol impurities from the previous step. The aqueous solution was then acidified with conc. HCl until the pH reached 2, and then was extracted with 3 x 400 mL of ether. The organic layer was dried with sodium sulfate, filtered by gravity and concentrated under reduced pressure. The residue was dried under high vacuum for several days. A viscous oil was collected which crystallized upon heating and triturating as a white waxy solid, 142.1 g (overall yield: 87%). NMR, δ (ppm), (100 MHz, $CDCl_3$) : 1.10 (s, 9H); 1.38 (d, J = 5Hz, 3H); 4.29 (q, J = 5Hz, 1H); 7.30 - 7.50 (m, 6H); 7.60 - 7.80 (m, 4H).

Synthesis of N-acetyl-S-(*t*-butyldiphenylsilyl)-(L)-lactoyl-(L)-cysteine, (5) To a solution of O-(*t*-butyldiphenylsilyl)-(L)-lactic acid (compound 4) (91.5 mmol, 20.04 g) in 100 mL of anhydrous THF was added, in four portions through a solid addition funnel, carbonyldiimidazole (109 mmol, 17.77 g). The addition of carbonyldiimidazole produced a significant volume of CO_2. The reaction mixture was stirred for 20 minutes until all the bubbling had stopped. N-Acetyl-(l)-cysteine (119 mmol, 19.5 g) was then added through a funnel in two portions while keeping the temperature of the reaction mixture from rising, via a water bath. Then 80 mL of THF was added to the reaction mixture to help dissolve all of the material. The reaction mixture was stirred for two hours (a thin layer chromatogram (tlc) sample showed complete conversion of the starting material). Half of the solvent was evaporated and 200 mL of water was added to the reaction mixture. The pH was lowered to two with conc. HCl. The product was then extracted with 3 x 250 mL of ethyl acetate. The organic layers were combined, dried over sodium sulfate, filtered by gravity, evaporated under reduced pressure, and pumped under high vacuum overnight. A white solid (42.91 g) was isolated. The tlc of the material showed more polar impurities. This material was then loaded on top of a column packed with 600 ml of silica for flash chromatography. The polarity of the eluant was increased from hexane:ethyl acetate:acetic acid (68:30:2) to hexane:ethyl acetate:acetic acid (48:50:2). Product 5 was isolated from condensed appropriate fractions in pure form 41.6 g

(96%). Tlc : eluant (hexane:ethyl acetate:acetic acid; 35:60:5); R_f : 0.91, 4; 0.27, 5, NMR, δ (ppm),(100 MHz, $CDCl_3$: 1.10 (s, 9H); 1.22 (d, J = 5Hz, 3H); 1.98 (s, 3H); 3.32 (d, J = 6 Hz, 2H); 4.35 (q, J = 5 Hz, 1H); 4.70 (q, J = 6 Hz, 1H); 5.6 - 6.0 (br. s); 6.42 (d, J = 6 Hz, 1H) 7.30 - 7.50 (m, 6H); 7.60 - 7.80 (m, 4H).

Synthesis of N-acetyl-S-(L)-lactoyl-(L)-cysteine (6) N-acetyl-S-(O-(t-butyldiphenylsilyl)-(L)-lactoyl)-(L)-cysteine (compound 5, 89.5 mmol, 42.4 g) was dissolved in 150 ml of THF. To this solution was added a commercially available THF solution of tetrabutyl ammonium fluoride, 1M (130 mmol, 130 mL). The reaction mixture was stirred for eight hours at room temperature. Thin layer chromatography indicated a complete conversion (eluant : chloroform:methanol:acetic acid; 85:10:5; R_f : 0.64, 5; 0.18, 6; 0.53, nBu_4N^+). The solvent was evaporated and the resulting orange oil was stirred vigorously into a suspension with 3 x 300 ml of ether to help remove most of the silanol by-product by decantation. The crude oil (59.2 g) was collected. This crude oil was dissolved in minimum dichloromethane and loaded on top of a column packed with 900 g of silica. The polarity of the eluant was increased from 100% dichloromethane to dichloromethane:methanol:acetic acid (93:5:2). Several fractions containing various amounts of tetrabutylammonium salt were collected and rechromatographed as described above. After four chromatographies, compound 6 was isolated as a glassy solid from

the purest fractions. Traces of acetic acid were removed by azeotropic distillation with cyclohexane. The remaining traces of organic solvents were removed by azeotropic distillation with water. The final material showed the presence of traces of tetrabutylammonium salt amounting to less than 2%, 11 g (52%). IR (cm^{-1}): 1725, 1690, 1640. NMR, δ (ppm),(100MHz, acetone d_6) : 1.32 (d, 3H); 1.93 (s, 3H); AB system : 3.12 (dd, 1H) and 3.45 (dd, 1H); 4.30 (q, 1H); 4.68 (m, 1H); 5.4 - 6.2 (br s); 7.42 (d, NH). Elemental analysis: $C_8H_{13}NO_5S$, % theory : C, 40.84; H, 5.57; N, 5.96; S, 13.6; % found: C, 41.35; H, 5.77; N, 5.77; S, 13.24.

Example 2 S-(N-Acetyl-(L)-cysteine) Derivative of Retinoic Acid

Synthesis of the S-(N-Acetyl-(L)-cysteine) derivative of retinoic acid (7) All trans-retinoic acid, (11.9 mmol, 3.58 g) was transferred into a dried flask wrapped in dark plastic cover to protect it from light. Under nitrogen, the trans-retinoic acid was dissolved in 60 mL of anhydrous THF. To this solution was added a solution of carbonyldiimidazole (15 mmol, 2.44 g) in 20 mL of anhydrous THF. The reaction mixture was stirred under nitrogen for 2.5 hours. The formation of the imidazolide was followed on thin layer chromatography (hexane:ethyl acetate:acetic acid (70:25:5); R_f : 0.49, retinoic acid, 0.38, imidazolide). When all the retinoic acid had been transformed to the imidazolide, N-acetyl-(L)-cysteine (17.36 mmol, 2.83 g) was added as a solid through a funnel under N_2. The reaction mixture

was stirred for 24 hours under N_2. The solvent was evaporated under vacuum at room temperature. The residue was dissolved in 200 mL of ethyl acetate and 120 mL of 0.1 N HCl was added to neutralize the imidazole released by the reaction. The aqueous phase was further extracted with 2 x 100 mL of ethyl acetate. The organic layers were combined, washed with 2 x 100 mL 10% NH_4Cl, dried over magnesium sulfate, filtered by gravity, and evaporated. A crude bright orange material was isolated (5.66 g), which was contaminated by traces of retinoic acid and some unknown polar compound (as seen on thin layer chromatography). The crude material was dissolved in minimum ethyl acetate and loaded on top of a column packed with thirty times the weight of crude product of silica in hexane:acetic acid (98:2). The polarity of the solvent was increased from hexane:acetic acid (98:2), to hexane:ethyl acetate:acetic acid (48:50:2). All the fractions were collected in the dark. Product 7 was eluted with a solvent polarity ranging from hexane:ethyl acetate:acetic acid (68:30:2) to hexane:ethyl acetate:acetic acid (48:50:2). The pure fractions containing 7 were combined and washed with 3 x 300 mL of water (until the pH reached 5) followed by 300 mL of brine, and then dried over sodium sulfate, and filtered by gravity. The solvent was evaporated under high vacuum with bath temperature not exceeding 30°C in a flask protected from light. NMR indicated a 1:1 mixture with ethyl acetate. This residual solvent could not be removed without heating above 30°C, and above this temperature, compound 7 decomposed.

Example 3 Synthesis of S-oleoyl-N-acetylcysteine

A dry 1000 mL, 3-neck, 24/40 round bottom flask equipped with an addition funnel, N_2 inlet, and magnetic stir bar, was filled with a solution of N-acetyl-L-cysteine (Aldrich, 8.77 g, 53.7 mmol) dissolved in 400 mL of dry tetrahydrofuran (THF, stored over molecular sieves-3A). Triethylamine (Aldrich, 5.73 g, 56.6 mmol) was added, and the reaction mixture chilled in an ice bath. Oleoyl chloride (Nu-Chek-Prep, 17.0 g, 56.4 mmol) was dissolved in 100 mL of THF and placed in the addition funnel. This solution was added dropwise over 0.5 hours. A white solid precipitated from the solution. The ice bath was allowed to melt, and the resulting suspension stirred at 25°C for 3 hours, by which time TLC (eluted with a mixture of ethylacetate and acetic acid) showed the reaction was nearly complete. The reaction mixture was poured into 1.0 liter of ethyl acetate and 0.5 liter of H_2O. The layers were separated, and the organic phase dried over $MgSO_4$, filtered, and concentrated to give an oil (approximately 25 g). This oil was combined with 1.3 g of nearly pure material from an earlier run and chromatographed on 370 g of silica gel, and then eluted with 75% EtOAc/Hexanes with 2% HOAc. Along with pure fractions, several impure fractions were obtained (4.8 g) which were repurified on 100 g of silica gel in the same eluent. All pure fractions were then combined, and concentrated (cyclohexane was used to azeotrope the acetic acid), and then pumped in for 4 days at 25°C, to constant weight. There was

obtained 12.08 g (47%) of 1 as a waxy off-white solid, homogeneous on TLC (10% MeOH/EtOAc, R_f= 0.42), mp 94-96°C.

Example 4 Regeneration of N-Acetylcysteine From S-Lauryl-N-acetylcysteine

For the N-acetylcysteine to be pharmaceutically active, the sulfhydryl group, which is protected as a thioester, must be regenerated by cleavage of the ester. Figure 1 illustrates the stability of S-lauryl-N-acetylcysteine after 1 and 24 hours at 37°C in an atmosphere of 5% CO_2, 95% air in a buffered solution consisting of 80% phosphate buffered saline (PBS), 20% methanol or in a solution that also contained fragments of fresh, surgically obtained, human skin. As is evident from the figure, the compound showed no signs of degradation during 24 hours in the buffered solution of PBS and methanol. In the presence of skin, however, a marked decrease in the concentration of the thioester was observed after 1 hour, and essentially no thioester remained in this solution after 24 hours. These results illustrate that human skin can promote transformation of S-lauryl-N-acetylcysteine to N-acetylcysteine and lauric acid.

IV. Method for Determining the Therapeutic Benefit of the Topical Application of Lipid Soluble Derivatives of N-Acetylcysteine in the Treatment and/or Prevention of Alopecia.

A number of animal model assays are available to evaluate the ability of a compound to treat alopecia. Any of these assays can be used to determine the ability of the compounds described herein to treat one or more forms of alopecia. Specifically, the ability of a thioester or thioether of N-acetylcysteine to enhance regrowth of hair or to minimize hair loss can be evaluated, for example, by one or more of the following methods:

(a) An established rodent (rat) model for chemotherapeutically induced alopecia where regrowth of hair can be measured/observed (see Example 5);

(b) an established mouse model which measures hair regrowth after androgenetic alopecia is induced (see Example 6);

(c) an established primate model (stumptail macaque monkey) in which the animals develop baldness in a similar pattern to androgenetic alopecia in humans (see Example 7); and

(d) in humans with alopecia caused by any of the factors outlined herein.

Example 5 Treatment of Alopecia Induced by Chemotherapeutic Agents: Rodent (Young Rat) Model

The systemic administration of cyclophosphamide, cytarabine or adriamycin chemotherapeutic agents to 8 day old rats induces hair loss in these young animals. The assay set

forth in Jiminez, J.J. , *et al.*, Cancer Investigation, 1992, Vol. 10(4), pp. 271-276 was used to assess the efficacy of the lipid soluble N-acetylcysteine prodrug in treating chemotherapeutically induced alopecia.

Lactating Fisher rats with 8-day old pups were purchased from Charles River Laboratories, Inc., Wilmington, MA. Rat pups used for each experiment were of the same age and weight ± 1 g. The chemotherapy dose was adjusted for individual animals. S-oleoyl-N-acetylcysteine (NACO) was synthesized according to the method described in Example 3.

All of the 8-day old Fisher rat pups received 30 mg/kg Cytoxan (cyclophosphamide in sterile saline) intraperitoneally in a single dose (Day 1). The rat pups were treated topically with approximately 0.5 mL of a 0%, 5%, or 10% solution of NACO in acetone once per day on their backs on Days 1 through 4 of the experiment. The rat pups were monitored daily through Day 10. All rats in the 0% (acetone alone) treated groups developed significant alopecia (hair loss) on their head and backs by Day 8. Rats in the 5% group were partially protected from the alopecia at the site of application, i.e., their backs, but unprotected from the hair loss on their heads. Rats in the 10% group were almost entirely protected from hair loss on their backs, but again were unprotected from the alopecia on their heads.

It is significant that the hair is lost on the heads of these animals in all groups. The local effect which we are

observing is desirable in the management of chemotherapeutically induced hair loss. These results demonstrate the local effect of applying the NACO, whereby only the treated areas are hairy and the untreated scalps are hairless as in the vehicle controls.

Example 6 Treatment of Androgenetic Alopecia: Mouse Model

A brief description of this model is outlined below (for details of the assay, see Matias, J.R. et al., Arch Dermatol Res. 1989, 281, 247-253).

The androchronogenic alopecia (AGA) mouse as a model for male-pattern baldness, with baldness being induced by the injection of testosterone or dihydrotestosterone. One group receives a topical application of the lipid soluble N-acetylcysteine derivative (e.g., S-lauryl-N-acetylcysteine), in a suitable vehicle several times daily. Another group receives topical application of vehicle control at the same dosing schedule. The degree of alopecia can then be measured. The effect of a topically applied lipid soluble thioester derivative of N-acetylcysteine in protecting mice from this form of alopecia may be limited to site of application or, if significant systemic absorption occurs, may result in a more generalized protection against hair loss and/or regrowth of hair.

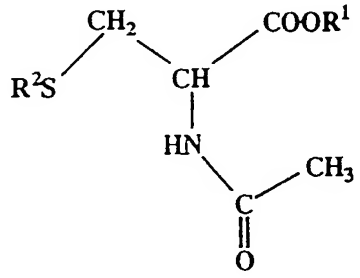
Example 7 Treatment of Androgenetic Alopecia: Macaque Monkey Model

The model disclosed by Rittmaster et al. (J. Clin. Endocrinol Metab., 1987, 65, 188-193) can be used to evaluate the

effectiveness of the N-acetylcysteine derivatives in the treatment of stump tailed macaque monkeys by replacing the N,N-diethyl-4-methyl-3-oxo-4-aza-5α-androstane-17β-carboxamide with the N-acetylcysteine derivatives.

We claim:

1. A method for the treatment of alopecia comprising the topical or transdermal administration to a mammal in need of such treatment of an effective amount of a compound of the formula:



or a pharmaceutical salt thereof,

wherein R^1 is selected from the group consisting of hydrogen, alkyl, aryl, alkaryl, aralkyl, alkoxyalkyl, aryloxyalkyl, an amino acid salt formed by the reaction of the amino group of a naturally occurring amino acid with the carboxylic acid group of the N-acetylcysteine or derivative thereof; an amine salt formed by the reaction of an amine-containing antibiotic with the carboxylic acid group of the N-acetylcysteine or an inorganic cation; and

R^2 is alkyl, aryl, aralkyl, alkaryl, alkyloxyalkyl, aryloxyalkyl, -C(O)-alkyl, -C(S)-alkyl, -C(O)-aryl, -C(S)-aryl, -C(O)-alkaryl, -C(S)-alkaryl, -C(O)-aralkyl, -C(S)-aralkyl, -C(O)-alkyloxyalkyl, -C(S)-alkoxyalkyl, -C(O)-acyloxyalkyl,

-C(S)-acyloxyalkyl, phosphate, the residue of a saturated or unsaturated fatty acid, the residue of an α-hydroxy acid, the residue of an alkyl dicarboxylic acid (where N-acetylcysteine is bound through either or both of the carboxylic acid groups), the residue of an aromatic dicarboxylic acid (where N-acetylcysteine is bound through either or both of the carboxylic acid groups), and the residue of another active molecule;

in a pharmaceutically acceptable carrier for topical administration.

2. The method of claim 1, wherein R^1 is selected from the group consisting of methoxymethyl, phenoxyethyl and an amino acid, wherein the amino acid is selected from the group consisting of alanyl, valinyl, leucinyl, isoleucinyl, prolinyl, phenylalaninyl, tryptophanyl, methioninyl, glycinyl, serinyl, threoninyl, cysteinyl, tyrosinyl, asparaginyl, glutaminyl, aspartoyl, glutaroyl, lysinyl, argininyl, and histidinyl; and

wherein R^2 is selected from methoxymethyl; phenoxymethyl; the residue of a saturated or unsaturated fatty acid selected from lauric, oleic, undecylinic, caproic, linoleic, linolenic, caprylic, capric, perlargonic, neononanoic, neodecanoic, palmitelaidoic, myristic, palmitic, stearic, arachidic, behenic, lignoceric, heptanoic, nonanoic, undecanoic, tridecanoic, pentadecanoic, heptadecanoic, nonadecanoic, heneicosanoic, tricosanoic, arachidonic, docosahexanoic, elaidic, erucic, nervonic, palmitoleic or petriselinic acid; the residue of lactic acid; the residue of retinoic acid, the residue of

ascorbic acid; the residue of an alkyl or aromatic dicarboxylic acid selected from azelaic acid, sebacic acid, phthalic acid, terephthalic acid, isophthalic acid, adipic acid, 1,10-dodecanoic acid, fumaric acid, 1,4-diphenylenediacrylic acid, azeleic acid, pimelic acid, suberic acid, itaconic acid, biphenyl-4,4'-dicarboxylic acid, benzophenone-4,4'-dicarboxylic acid, p-carboxyphenoxyalkanoic acid, hydroquinone-O,O-diacetic acid, 2,2-bis-(4-hydroxyphenyl)propane-O,O-diacetic acid, 1,4-phenylene-dipropionic acid, and cyclohexane dicarboxylic acid; methotrexate; bis(p-carboxyphenoxyalkane); 1,4-bis-carboxymethyl benzene; cromolyn; nedocrimil; 1,3,5-benzenetricarboxylic acid; and the residue of another active molecule selected from vitamin D and derivatives thereof, and vitamin E and derivatives thereof, where the formation of any number of thioether or ester bonds is possible.

3. The method of claim 2, wherein the active molecule is selected from 1α,25-dihydroxy vitamin D_3, (1α,24,25)-trihydroxy vitamin D_3, 1α-hydroxy vitamin D_3, (1α,25,26)-trihydroxy vitamin D_3 and derivatives thereof, wherein the derivatives are the hydroxylated, alkylated, and acylated derivatives.

4. The method of claim 1 wherein the compound is administered several times a day.

5. The method of claim 1 wherein R^1 is an amino acid.

6. The method of claim 5 wherein the amino acid is selected from the group consisting of lysine and arginine.

7. The method of claim 1 wherein R^1 is an amine-containing antibiotic.

8. The method according to claim 7 wherein the antibiotic is selected from the group consisting of erythromycin, propionylerythromycin, neomycin, gentomycin, tobramycin, kanamycin, and mechlocycline.

9. The method of claim 1 where R^1 or R^2 is an antioxidant.

10. The method of claim 9 where the antioxidant is selected form the group consisting of ascorbic acid or vitamin E or derivatives.

11. The method of claim 1 where R^1 or R^2 is a free radical scavenger.

12. The method of claim 1, wherein R^1 or R^2 is a fatty acid residue.

13. The method of claim 12 wherein the fatty acid residue is selected from the group consisting of lauric, oleic, caproic, linoleic, linolenic, caprylic, capric, myristic, palmitic, stearic, arachidic, behenic, lignoceric, heptanoic, nonanoic, undecanoic, tridecanoic, pentadecanoic, heptadecanoic, nonadecanoic, heneicosanoic, tricosanoic, arachidonic, docosahexanoic, elaidic, erucic, nervonic, palmitoleic or petriselinic acid.

14. The method of claim 13 where the acid residue is from the group consisting of lauric or oleic acid.

15. The method of claim 1 wherein the mammal is a human.

16. The method of claim 1 wherein the compound is administered in a concentration between 0.001 and 50 mg/kg/day.

17. The method of claim 1 wherein the compound is administered in a controlled release formulation.

18. The method of claim 1, the compound is administered in combination with another compound or compounds selected from the group consisting of antivirals, antibiotics, anti-inflammatories, and immunosuppressants.

19. The method of claim 1, wherein the compound also has anti-inflammatory activity.

20. The method of claim 1, wherein the compound also has immunosuppressive activity.

21. The method of claim 1, wherein the compound also has antioxidant activity.

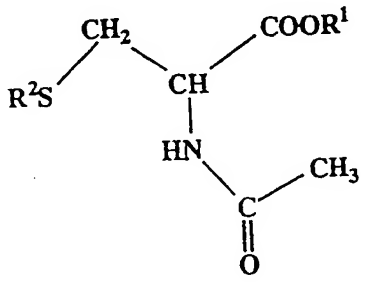
22. The method of claim 1, wherein the compound also has free radical scavenging activity.

23. The method of claim 1, wherein the compound is selected from the group consisting of (S-oleoyl-N-acetyl-L-cysteine), (S-lauryl-N-acetyl-L-cysteine, (S-myristoyl-N-acetyl-L-cysteine), (S-caprolyl-N-acetyl-L-cysteine), (S-3,7-dimethyl-9-(2,6,6-trimethyl-1-cyclohexen-1-yl)-2,4,6,8-nonatetraenolyl-N-acetyl-L-cysteine), (S-ascorboyl-N-acetyl-L-cysteine) and (S-lactoyl-N-acetyl-L-cysteine).

24. The method of claim 1 wherein the compound is administered topically.

25. The method of claim 1, wherein the inorganic cation is selected from the group consisting of sodium, potassium, magnesium, calcium, zinc, bismuth, barium, aluminum, copper, cobalt, nickel, and cadmium.

26. A topical composition for the treatment of alopecia comprising an effective amount of a compound of the formula:



or a pharmaceutically acceptable salt thereof;

wherein R^1 is selected from the group consisting of hydrogen, alkyl, aryl, alkaryl, aralkyl, alkoxyalkyl, aryloxyalkyl, an amino acid salt formed by the reaction of the amino group of a naturally occurring amino acid with the carboxylic acid group of the N-acetylcysteine or derivative thereof; an amine salt formed by the reaction of an amine-containing antibiotic with the carboxylic acid group of the N-acetylcysteine or an inorganic cation; and

R^2 is alkyl, aryl, aralkyl, alkaryl, alkyloxyalkyl, aryloxyalkyl, -C(O)-alkyl, -C(S)-alkyl, -C(O)-aryl, -C(S)-aryl,

-C(O)-alkaryl, -C(S)-alkaryl, -C(O)-aralkyl, -C(S)-aralkyl, -C(O)-alkyloxyalkyl, -C(S)-alkoxyalkyl, -C(O)-acyloxyalkyl, -C(S)-acyloxyalkyl, phosphate, the residue of a saturated or unsaturated fatty acid, the residue of an α-hydroxy acid, the residue of an alkyl dicarboxylic acid (where N-acetylcysteine is bound through either or both of the carboxylic acid groups), the residue of an aromatic dicarboxylic acid (where N-acetylcysteine is bound through either or both of the carboxylic acid groups), and the residue of another active molecule;

or a pharmaceutically acceptable salt thereof;

in a pharmaceutically acceptable carrier for topical administration.

27. The composition of claim 26, wherein R^1 is selected from the group consisting of methoxymethyl, phenoxyethyl and an amino acid, wherein the amino acid is selected from the group consisting of alanyl, valinyl, leucinyl, isoleucinyl, prolinyl, phenylalaninyl, tryptophanyl, methioninyl, glycinyl, serinyl, threoninyl, cysteinyl, tyrosinyl, asparaginyl, glutaminyl, aspartoyl, glutaroyl, lysinyl, argininyl, and histidinyl; and

wherein R^2 is selected from methoxymethyl; phenoxymethyl; the residue of a saturated or unsaturated fatty acid selected from lauric, oleic, caproic, linoleic, linolenic, caprylic, capric, perlargonic, neononanoic, neodecanoic, palmitelaidoic, myristic, palmitic, stearic, arachidic, behenic, lignoceric, heptanoic, nonanoic, undecanoic, tridecanoic,

pentadecanoic, heptadecanoic, nonadecanoic, heneicosanoic, tricosanoic, arachidonic, docosahexanoic, elaidic, erucic, nervonic, palmitoleic or petriselinic acid; the residue of lactic acid; the residue of retinoic acid, the residue of ascorbic acid; the residue of an alkyl or aromatic dicarboxylic acid selected from azelaic acid, sebacic acid, phthalic acid, terephthalic acid, isophthalic acid, adipic acid, 1,10-dodecanoic acid, fumaric acid, 1,4-diphenylenediacrylic acid, azeleic acid, pimelic acid, suberic acid, itaconic acid, biphenyl-4,4'-dicarboxylic acid, benzophenone-4,4'-dicarboxylic acid, p-carboxyphenoxyalkanoic acid, hydroquinone-O,O-diacetic acid, 2,2-bis-(4-hydroxyphenyl)propane-O,O-diacetic acid, 1,4-phenylene-dipropionic acid, and cyclohexane dicarboxylic acid; methotrexate; bis(p-carboxyphenoxyalkane); 1,4-bis-carboxymethyl benzene; cromolyn; nedocrimil; 1,3,5-benzenetricarboxylic acid; and the residue of another active molecule selected from vitamin D and derivatives thereof, and vitamin E and derivatives thereof, where the formation of any number of thioether or ester bonds is possible.

28. The composition of claim 27, wherein the active molecule is selected from 1α,25-dihydroxy vitamin D_3, (1α,24,25)-trihydroxy vitamin D_3, 1α-hydroxy vitamin D_3, (1α,25,26)-trihydroxy vitamin D_3 and derivatives thereof, wherein the derivatives are the hydroxylated, alkylated, and acylated derivatives.

29. The composition of claim 26, in the form of a topical solution, gel, ointment, cream, lotion or foam.

30. The composition of claim 26 in the form of a gauze soaked bandage.

31. The composition of claim 26 wherein R^1 is an amino acid.

32. The composition of claim 31, wherein the amino acid is selected from the group consisting of lysine and arginine.

33. The composition of claim 26, wherein R^1 is an amine containing antibiotic.

34. The composition of claim 33, wherein the antibiotic is an selected from the group consisting of erythromycin, propionylerythromycin, neomycin, gentomycin, tobramycin, kanamycin and mechlocycline.

35. The composition of claim 26, wherein R^2 is a fatty acid residue.

36. The composition of claim 35, wherein the fatty acid is selected from the group lauric, oleic, caproic, linoleic, linolenic, caprylic, capric, myristic, palmitic, stearic, arachidic, behenic, lignoceric, hexanoic, nonanoic, undecanoic, heneicosanoic, tricosanoic, arachidonic, docosahexanoic, elaidic, erucic, nervonic, palmitoleic, or petriselinic acid.

37. The composition of claim 26 where R^1 or R^2 is an antioxidant.

38. The composition of claim 37 where the antioxidant is selected form the group consisting of ascorbic acid or vitamin E or derivatives thereof.

39. The composition of claim 26 in a controlled release formulation.

40. The composition of claim 26, wherein the compound is administered in combination with another compound or compounds selected from the group consisting of antivirals, antifungals, antibiotics, anti-inflammatories, anti-oxidants, and immunosuppressants.

41. The composition of claim 26, wherein the compound has anti-inflammatory activity.

42. The composition of claim 26, wherein the compound has immunosuppressive activity.

43. The composition of claim 26, wherein the compound has antioxidant activity.

44. The composition of claim 26, wherein the compound has free-radical scavenging activity.

45. The composition of claim 26, wherein the compound is selected from the group consisting of (S-oleoyl-N-acetyl-L-cysteine), (S-lauryl-N-acetyl-L-cysteine), (S-myristoyl-N-acetyl-L-cysteine), (S-caprolyl-N-acetyl-L-cysteine), (S-3,7-dimethyl-9-(2,6,6-trimethyl-1-cyclohexen-1-yl)-2,4,6,8-nonatetraenoyl-N-acetyl-L-cysteine), (S-ascorboyl-N-acetyl-L-cysteine) and (S-lactoyl-N-acetyl-L-cysteine).

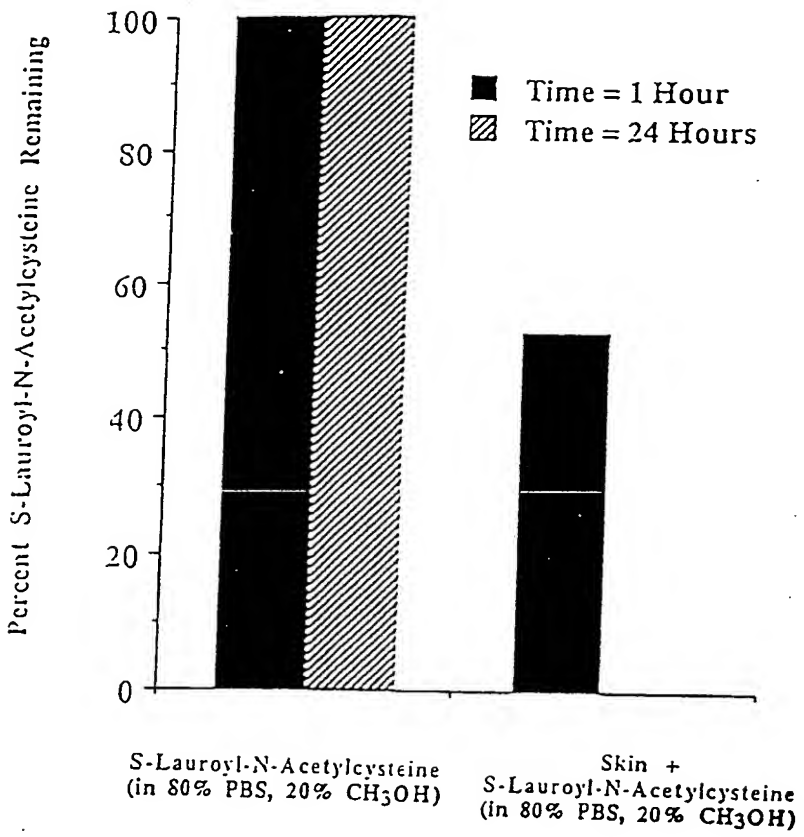

Percent S-Lauroyl-N-Acetylcysteine Remaining
100
80
60
40
20
0
Time = 1 Hour
Time = 24 Hours
S-Lauroyl-N-Acetylcysteine (in 80% PBS, 20% CH3OH)
Skin + S-Lauroyl-N-Acetylcysteine (in 80% PBS, 20% CH3OH)

Figure 1

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/07470

A. CLASSIFICATION OF SUBJECT MATTER
IPC(6) :A61K 31/23
US CL :514/550, 562
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/550, 562

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, CAS ONLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US, A, 5,296,500 (HILLEBRAND) 22 March 1994, column 3, lines 1-55; column 6, lines 49-50; column 17, claim 1; column 18, claim 10.	26, 29, 40-41, 43-44.
X	US, A, 4,567,163 (PONCHIROLI) 28 January 1986, column 4, claims 1; 3-6.	26, 31-34
X --- Y	Cancer Investigation, volume 10, No. 4, issued April 1992, Jimenez et al., "Treatment with ImuVert/N-acetylcysteine protects rats from cyclophosphamide/cytarabine-indufed alopecia", pages 271-276. See abstract.	1-14, 16-25

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 27 JULY 1995	Date of mailing of the international search report 12 OCT 1995
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer EVELYN HUANG Telephone No. (703) 308-1235

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/07470

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Cellular Immunology, Volume 140, issued 1992, P. Peristeris et al., "N-acetylcysteine and flutathione as inhibitors of tumor necrosis factor production", page 390-399. See abstract.	20
Y	Pharmacology & Toxicology , Volume 66, issued 1990, T. Lalitha et al., "Effect of N-acetyl-cysteine, D-Penicilllamine and buthionine dulfoximine on Glutathione levels and CNSoxygen toxicity in rats", page 56-61. See abstract.	21-22
X	EP, A, 0,415,598 (UNILEVER PLC) 06 March 1991. See abstract. Pages 14-15, Examples 12, 13.	1-17, 19-25
Y	H. Bundgarrd, "Design of Prodrugs", published 1985 by Elsevier (Amsterdam), see pages 1-3 and 79, especially pages 1 and 79.	1-14, 16-25